Long-term effects of Combat Ready Clamp application to control junctional hemorrhage in swine

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BACKGROUND: Groin application of Combat Ready Clamp (CRoC) in pigs elicits an acute inflammation in underlying ischemic tissues. This study examined functional recovery of pigs' hind leg(s) following 2 hours of CRoC application.

METHODS: Left femoral arteries were isolated and injured in anesthetized pigs. Following 25% hemorrhage, CRoC was applied on the inguen for 2 hours (n 6), and wounds were covered with combat gauze (CG). Bleeding was treated in the control animals (n 5) with CG only. Next, CRoC and CG were removed, arteries were repaired and reflowed, and animals were recovered. The legs' mobility was scored daily, and their neuromuscular functions were measured on Days 7 and 14. Computed tomographic angiography and blood analysis were performed on Days 0, 2, 7, and 14. Pigs were then euthanized, and tissues were collected for histology. Umbilicus application of CRoC was also tested in four pilot experiments.

RESULTS: Inguinal application of CRoC with 524 ± 12 mm Hg pressure occluded iliac arteries and collateral circulation. Following surgical repair, blood flow to the arteries was restored, and five of six CRoC-applied legs recovered full mobility within 9 days. Control-treated legs recovered full function in 3 days (p 0.001). At 2 weeks, muscle strength of CRoC-applied legs was diminished ($p < 0.05$ vs. baselines or controls). Injury biomarkers in the CRoC group increased severalfold compared with the controls on Day 2 but returned to baseline afterward. Histologic changes were mostly mild and indicative of ischemia in the CRoC group. Umbilical application of CRoC required higher pressure (625 ± 8 mm Hg) and caused gross ischemic necrosis of lumbar muscles with significant disabilities.

CONCLUSION: Two-hour inguinal application of CRoC caused mild and reversible ischemic injuries, which delayed full recovery of the limb function by a few days. In contrast, 2-hour umbilicus application of CRoC resulted in extensive muscle necrosis with functional disabilities. While CRoC seems safe and effective for inguinal application, other tourniquets should be evaluated for treating bilateral junctional bleeding. (*J Trauma Acute Care Surg*. 2014;77: S101 S108. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Combat Ready Clamp; hemorrhage control; junctional bleeding; junctional tourniquet; swine.

Widespread use of tourniquets (TQs) and hemostatic dressings on the battlefield has reduced mortalities from isolated extremity injuries. Consequently exsanguination from junctional wounds, which cannot be controlled by either method, has become common and lethal in the ongoing conflict.[1,2] This rise of death from junctional injuries has prompted the development of several new TQs specialized to stop junctional bleeding. The first device, Combat Ready Clamp (CRoC), received Food and Drug Administration (FDA) clearance in 2010 for treating difficult inguinal hemorrhage and has been sent to the battlefield for use. CRoC indication was expanded for the application on other anatomic sites such as umbilicus and subclavian areas to control bilateral groin and axilla hemorrhage, respectively. Other junctional TQs were later developed and received clearance for treating inguinal bleeding. These include Abdominal Aortic TQ, Junctional Emergency Treatment Tool, and SAM Junctional TQ. The supporting efficacy data to receive FDA clearance for these devices were mostly collected from perfused human cadaver studies.[3] The feasibility and comfort of some devices were also tested in a small number of healthy volunteers.[4] There are a few anecdotal reports indicating efficacy of these devices.[5,6]

Previously, we conducted the first proof-of-concept study examining the effectiveness of CRoC to control a groin hemorrhage in swine.[7] Our results showed that CRoC is an effective hemostatic adjunct and, combined with ordinary gauze, can stop lethal hemorrhage and maintain hemostasis when applied on the wound. However, to achieve hemostasis, CRoC required high pressure occluding both the injured femoral and the larger iliac arteries and preventing collateral circulation in the limb. Histologic examination of affected tissues showed focal inflammation of the femoral vein and perineural sheath of the saphenous nerve with unknown clinical consequences. These acute histologic changes were seen even in the absence of blood reperfusion. Therefore, this study was performed to explore long-term effects of ischemic-reperfusion injuries associated with CRoC application on swine limb function.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. It was conducted in compliance with the Animal Welfare Act and implemented Animal Welfare Regulations. All animals received

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This article is not an endorsement of the CRoC by the authors or the US Army but is simply a report of our observations with experimental use of the device.
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care and were used in strict compliance with *The Guide for the Care and Use of Laboratory Animals*.[8]

Yorkshire female cross-bred pigs (45–55 kg) were purchased from Midwest Research Swine (Gibbon, MN) and housed for 1 week to allow acclimation and to test for any preexisting disease. Pigs were fasted for 12 hours to 18 hours before surgery with free access to water. On the day of the surgery, pigs were induced, intubated, anesthetized (1–2% isoflurane), mechanically ventilated, and prepared for sterile operation as described previously.[9] Maintenance fluid (lactated Ringer's solution) was administered at 5 mL/kg per hour intravenously. Body temperature was monitored and maintained at approximately 38°C.

Experimental Design

The study was design to include three arms: the first arm was to investigate the long-term effect of the application of CRoC on the inguen for unilateral control of junctional hemorrhage; the second arm was a control treatment to determine the long-term effect of the use of combat gauze (CG) for treating similar junctional bleeding; and the third arm was designed to explore the long-term effect of the application of CRoC on the umbilicus for bilateral control of junctional hemorrhage. In addition to these arms, a few model development experiments were initially performed, and also, a small pilot experiment was conducted to assess the feasibility of applying CRoC on the pigs' umbilicus to control junctional hemorrhage.

Surgical Procedures

A venous catheter (14 gauge) was introduced percutaneously into the external jugular vein and secured externally for chronic use. A small (1.3-mm diameter) gel-filled catheter, attached to a precalibrated transducer (Data Scientifics International, St. Paul, MN) was placed in a branch of the right femoral artery. Vital signs (blood pressures and heart rate) were received and recorded by a computer system. Next, an incision was made on the inner thigh, and approximately 3 cm of femoral artery was isolated. After a 10-minute stabilization, the artery was partially transected (approximately 50% of circumference), and uncontrolled hemorrhage was allowed until approximately 25% of the circulating blood volume was lost. The shed blood was continuously collected and weighed to estimate blood loss. Before the start of the study, four model development experiments were performed to examine the overall effects of isolation and injury of the femoral artery, a 25% uncontrolled hemorrhage, and a subsequent repair of the vessel (without applying CRoC or gauze with minimum ischemic time) on the general recovery and mobility of the animals. In the first arm of the study following hemorrhage, CRoC was assembled and placed on the inguinal area (above the wound) and tightened until bleeding stopped (n = 6). Minor oozing was still present in the wound, which was treated by placing a CG in the wound. In the second arm of the study (the control animals), bleeding was controlled solely by packing the wound with CG (n = 5). Animals then received limited fluid resuscitation (500–800 mL of Hextend) to a target mean arterial pressure of 60 mm Hg and were monitored for 2 hours.

Afterward, CRoC and CG were removed, and the artery was temporarily clamped. The vascular cut was then sutured (7–0 Prolene, interrupted), blood flow was restored, and the groin wound was closed in layers (no anticoagulant injected). The blood pressure catheter was also removed, and the vessel was repaired. Animals were recovered from anesthesia and evaluated for the next 2 weeks using tests described later. Pigs received adequate analgesia (transdermal fentanyl patch and hydromorphone injections) for a few days to prevent postoperative pain. At the end of the surgery, the compression pressure generated by CRoC was measured in all but two animals (n = 4) by reapplying CRoC briefly with a pneumatic cuff placed on the skin beneath the pressure disk. The disk was then tightened to the same level as before, and the generated pressure was read on a digital manometer. Performing this measurement during the surgical procedure would have contaminated the surgical field and could have caused wound infection, thereby compromising the results.

The third arm of the study was designed to explore long-term effects of a 2-hour application of CRoC on the lower abdomen (umbilicus) to occlude the abdominal aorta at the bifurcation site and stop blood flow in both lower extremities. This was consistent with a new FDA-approved indication of CRoC for the control of bilateral hemorrhage in casualties with double amputees. Before the start of this arm of the study, four pilot experiments were performed to examine the feasibility of applying CRoC on the pig's umbilicus and stopping blood flow successfully in both lower legs. The pressure generated by the CRoC was measured in these pigs as described before. These pilot experiments, which were followed for 2 weeks (in three animals), revealed significant disability in the animals to the extent that experimentation in this arm of the study was suspended. The results of the pilot experiments are presented here to report potential damaging effects of umbilicus CRoC application.

Gait and Posture Assessment

This was performed by two veterinary technicians who were blinded to the treatments. Before the anesthesia induction (to measure baseline) and following the surgery, animals were carefully observed each day, weighed, and encouraged to walk for scoring according to the modified Tarlov scale (Fig. 1 legend).

Neuromuscular Function Measurement

On Day 0 (before the start of surgical procedure for baseline measurement) and Days 7 and 14 after the operation, the anterior crural muscle (tibialis, digitorum, peroneous tertias) strength was measured in vivo using a large animal force transducer (890A, Aurora Scientific, Ontario, Canada). While under anesthesia, the foot of the injured leg was attached to the foot plate of the force transducer with the foot plantar flexed and the knee at a right angle. Maximal isometric tetanic torque was elicited by stimulating (80–100 Hz, 0.1-millisecond pulse, 800-millisecond train) the peroneal nerve with percutaneous needle electrodes.

Computed Tomographic Scans and Blood Analysis

In addition to baseline and postoperative blood samples, on Days 2, 7, and 14, animals were reanesthetized, venous blood samples were collected, and computed tomographic (CT) angiography was performed. Images of blood flow through the affected arteries were obtained after an intravenous contrast injection (100 mL, Omnipaque). Blood samples were analyzed for complete blood cell count, blood gases, coagulation (prothrombin time, activated partial thromboplastin time, and fibrinogen) and

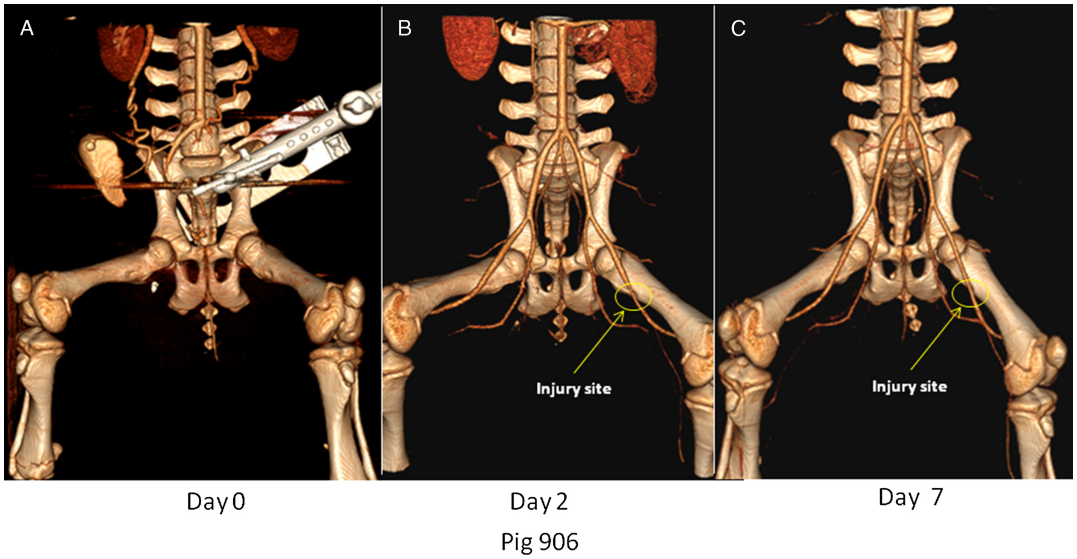

Figure 1. CT images of lower body arterial circulation of a CRoC-treated pig. *A*, Inguinal application of CRoC, with sufficient pressure to stop groin hemorrhage, occluding blood flow through iliac arteries and collateral vessels (total ischemia). *B*, The Day 2 image reveals restoration of blood flow through the arteries with some restriction (stenosis) in the femoral artery at the injury site. *C*, The Day 7 image shows normal blood flow in both hind legs.

selected chemistry parameters, which included tissue injury biomarkers such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST).

Tissue Collection for Histology

On Day 14 after administering anesthesia, groin wounds were reopened, and the repaired segment of femoral artery with adjacent femoral vein and saphenous nerve were recovered and fixed for histology. The peroneus tertius muscle in which contractile strength had been measured and its peroneal nerve were also recovered for histology. The animal was then euthanized by an injection, and small segments of the sciatic nerve and spinal cord at L3 level were also collected for histologic evaluation. Histologic slides, stained with hematoxylin and eosin, were examined by a board-certified veterinarian pathologist (J.S.E.) who was blinded to the sample treatment until all samples were individually analyzed.

Data Analysis

Data are expressed as mean ± SEM. Blood test results were analyzed by Mann-Whitney (two-tailed) U-test. The daily gait scores were compared using two-way Friedman test, and the heart rate changes were tested using two-way repeated-measures analysis of variance. A $p < 0.05$ was considered statistically significant.

RESULTS

Main Study (Inguinal Applied CRoC vs. Control Animals)

Baseline measurements including vital signs and blood test results were within normal range for all subjects, with no difference between the two groups. The average pretreatment blood loss was 17.1 ± 0.5 mL/kg for the CRoC and 16.9 ± 0.7 mL/kg for the control group or approximately 25% of their blood volume. CRoC was partially assembled and positioned before the start of the experiment, but an additional 2.6 ± 0.3 minutes was needed to complete assembly and application. Packing the wound with CG in the control group took approximately 1.9 ± 0.2 minutes. The approximate CRoC pressure on the inguinal region was 524 ± 12 mm Hg, which was measured at the conclusion of the surgical procedure (after closing skin) by reapplying the CRoC in the same way as before to avoid contamination of sterile surgical field. We were unable to sterilize the delicate plastic pneumatic cuff and tubing that were used to measure CRoC pressure. Blood pressure (mean arterial pressure) remained stable (60–65 mm Hg) during hemostatic treatment in both groups following fluid resuscitation (approximately 700-mL Hextend). Heart rate, however, increased on average from 78 ± 5 beats per minute to 119 ± 8 beats per minute during the 2 hours of CRoC application, which was significantly faster than the increase measured in the control animals (80 ± 5 beats per minute to 96 ± 6 beats per minute, $p < 0.0001$).

CT Scans and Blood Test Results

CT images of arterial vasculature showed complete occlusion by CRoC (Fig. 1*A*) and patent arteries with stenosis on the injured site at Day 2 (Fig. 1*B*). By Day 7, stenosis was resolved, and normal blood flow through the artery was restored in both groups (Fig. 1*C*). Blood gas analysis of venous blood samples showed no significant changes in shock indices (i.e., base excess, lactate, pH) postoperatively and in the subsequent measurements. Changes in red blood cell, white blood cell, and platelet counts were similar in both groups (data not shown). K^+concentration significantly increased in the CRoC pigs postoperatively but normalized on Day 2 (Fig. 2*A*). Other tissue injury biomarkers (CPK, LDH, and AST) were severalfold higher in the CRoC pigs than in the control animals on Day 2 but returned to baseline concentrations on Day 7 (Fig. 2*B–D*).

Hind Leg Functional Recovery

Control animals were able to stand on their hind legs shortly after operation and gained full function of their hind legs (Tarlov score, 4) by Day 3 (Fig. 3). CRoC-treated animals however showed greater disability early after operation, and the complete recovery of their treated legs was delayed ($p = 0.001$ vs. the controls). Five of six pigs in this group gained full leg function by Day 9, but one pig did not recover normal mobility in the 2-week period (Tarlov score, 3). The weight of the animals increased by 8% (controls) to 10% (CRoC) during the observation period and had similar pattern.

Before surgery, in vivo maximal anterior crural muscle torque production was approximately 0.61 Newton meter per kilogram of body weight, with no difference between the groups. Muscle strength was measured again on Day 7 and was found to be reduced by 15% (controls) and 28% (CRoC). On the Day 14 measurement, control muscles completely recovered, but the CRoC muscles remained weak, with 32 % less strength than baseline and in comparison with controls ($p < 0.05$, Mann Whitney U-test; Fig. 4*A* and *B*).

Vessel, Nerve, and Muscle Histology

During necropsy, no gross lesions were observed. Femoral arteries and veins collected from wounds were equally affected in the CRoC-treated and control groups and showed only normal regenerative changes. Muscle (peroneus tertius) fiber necrosis was not seen in the controls (Fig. 4*C*) but was found in three of six CRoC-treated animals including one severe case (Fig. 4*D*).

Perineural sheath proliferation was found in saphenous and sciatic nerves of 50% of the CRoC animals but seen only in 20% of the control pigs (Fig. 5*A* and *B*, normal vs. affected). These reactive changes most likely had no affect on nerve conduction. Axonal degeneration was observed in sciatic nerves of 50% of the CRoC group (Fig. 5*C* and *D*, normal vs. affected), but it was seen together in the same animal and most likely resulted from ischemic insult.

Umbilicus Application of CRoC (Pilot Experiments)

Application of CRoC on the umbilicus was more difficult than applying CRoC on the inguen. Umbilicus application required several locational adjustments and more tightening to produce the higher pressure (625 ± 8 mm Hg) needed to occlude blood flow in both hind legs. Three animals in which blood flow was successfully blocked by CRoC for 2 hours developed significantly more disabilities than seen in the inguinal CRoC group. One animal showed no mobility improvement 3 days after surgery (scored 1) and had to be euthanized. This pig was also unable to void and had excessively full bladder during this period. When this animal and another pig that did not fully recover in 2 weeks underwent necropsy, deep and widespread necroses were found on their lumbar muscles, which

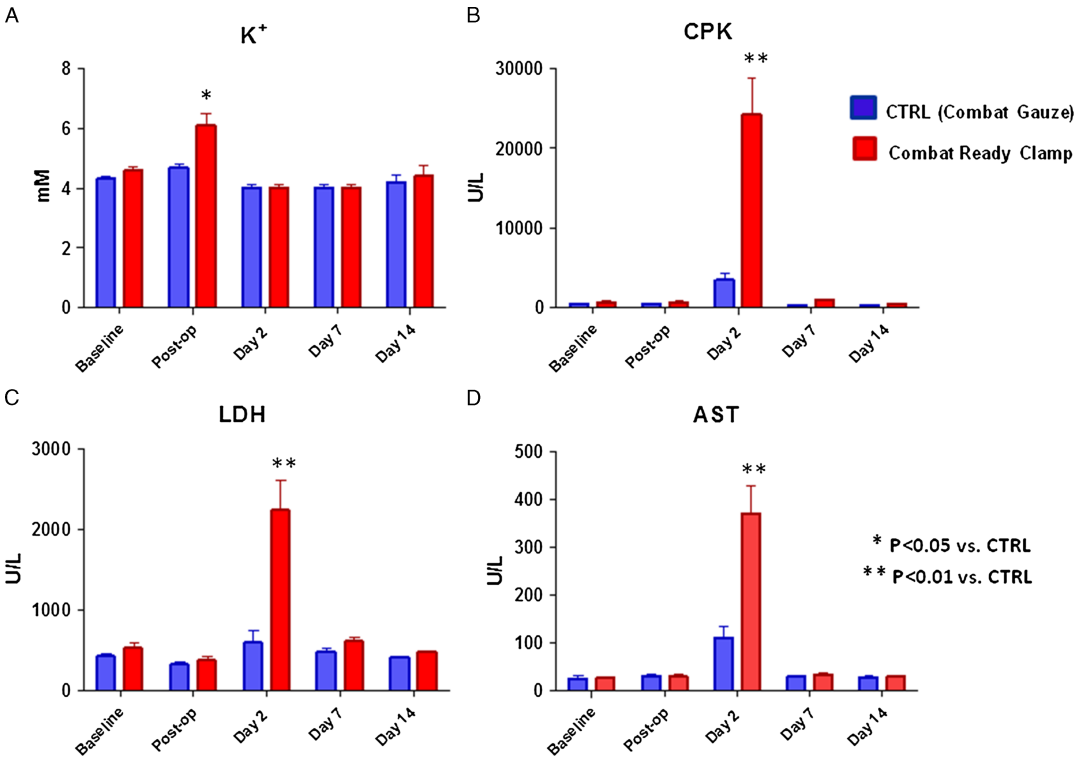

Figure 2. Circulating tissue injury biomarkers measured in blood samples. Inguinal application of CRoC for 2 hours was associated with significant increases of K^+ concentration postoperatively (*A*) and CPK (*B*), LDH (*C*), as well as AST (*D*) during 2 days after the operation. All these biomarkers returned to baseline levels within 1 week after the surgery. Data are expressed as mean ± SEM and analyzed by Mann-Whitney U-test (n = 5 6 per group).

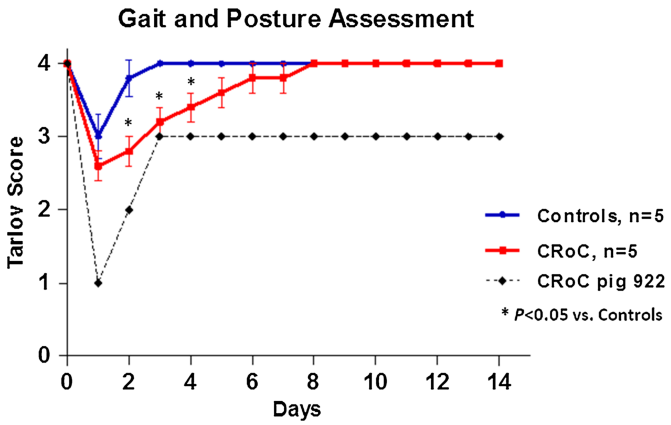

Figure 3. Gait and posture assessments of inguinal-applied pigs following the operations. Pigs were observed daily and scored according to a modified Tarlov scale. The scores were 0 (insensate and paralyzed), 1 (only able to sit), 2 (stands but unable to bear any weight on affected leg), 3 (stands and walks but with abnormal gait and posture), and 4 (normal posture and gait). Control animals had full clinical recovery within 3 days (score, 4), while five of six CRoC-treated (inguinal) animals had delayed recovery lasting up to 9 days to complete ($p = 0.001$). One CRoC animal (Pig 922) did not fully recover (Tarlov score, 3) during the 2-week observation period.

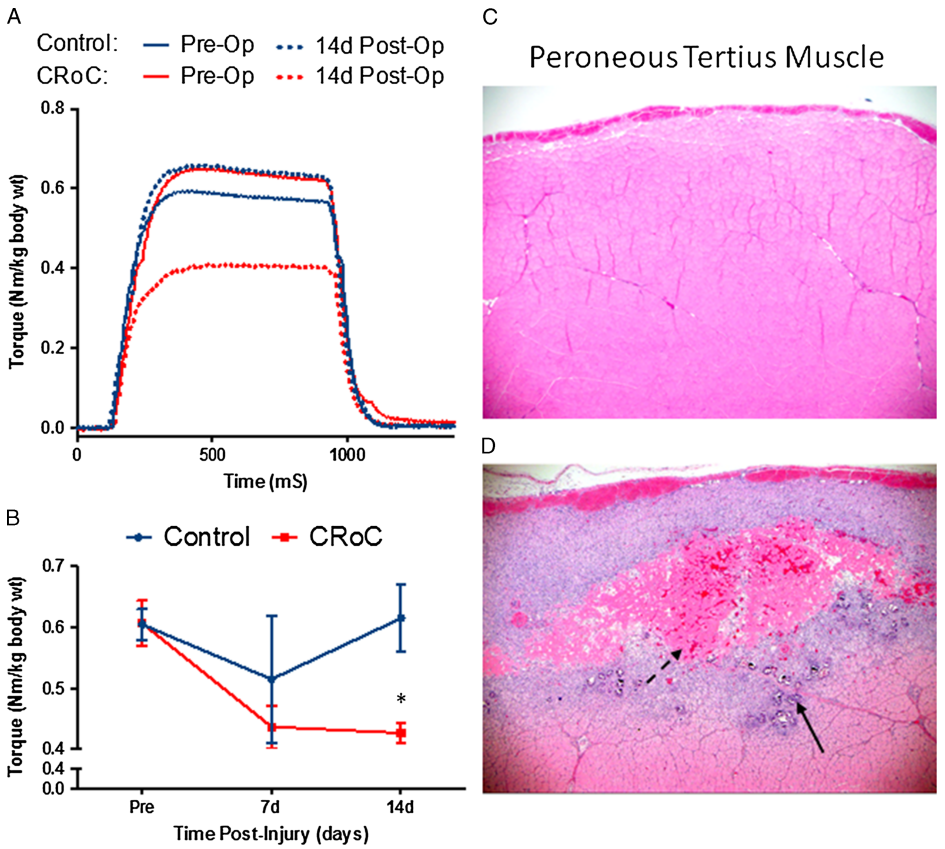

Figure 4. Anterior crural muscle in vivo strength assessment and histology of inguinal-applied CRoC pigs. (*A*), Digitized maximal isometric torque wave forms measured before and Day 14 after injury are presented for representative control and CRoC pigs. (*B*), Isometric torque group measured before, 7 days, and 14 days after injury (*$p < 0.05$ vs. the controls). Micrographs of the primary anterior crural muscle, peroneus tertius (original magnification 4×) are presented from a control (*C*) and a CRoC-treated pig (*D*) that did not fully recover gait and posture or in vivo functional capacity by Day 14 after injury (Pig 922). Note central muscle necrosis (*dashed arrow*) and fibrosis and mineralization of surrounding tissues (*solid arrow*) in the slide.

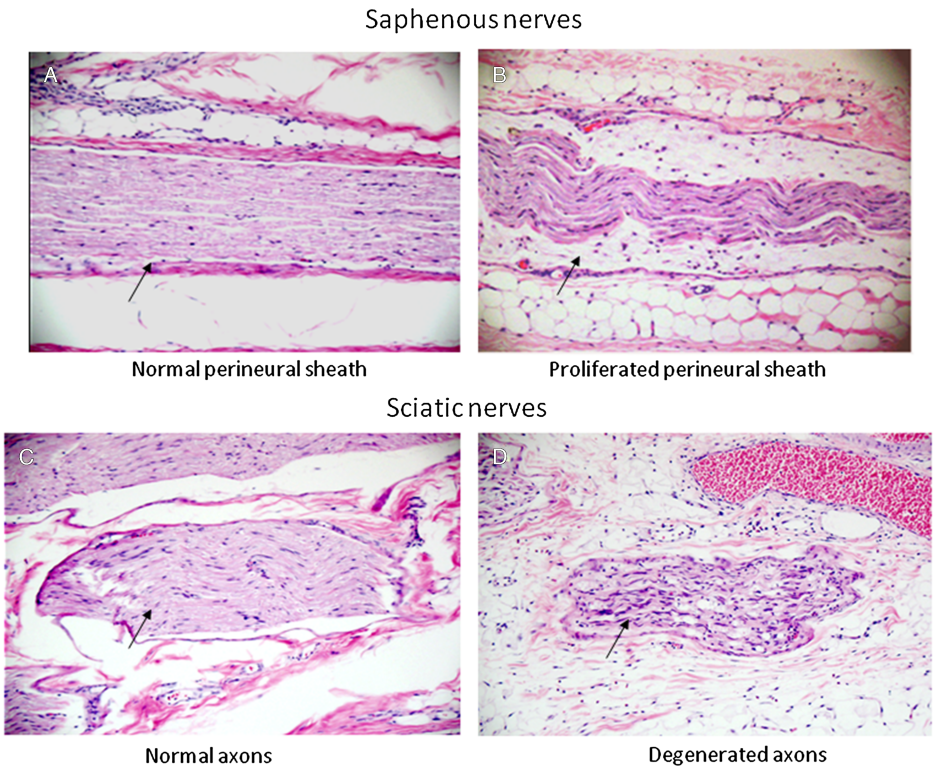

Figure 5. Histologic changes of saphenous and sciatic nerves (proliferation of perineural sheath and axonal degeneration, original magnification 20×) seen in both the control and CRoC-treated (inguinal application) samples. These reactive changes were more common in CRoC than in control animals and were likely caused by ischemia.

could only be explained by total and permanent ischemia (Fig. 6). Further experimentation of this group was therefore halted.

DISCUSSION

This study was performed to explore potential long-term adverse effects of using CRoC to temporarily control unilateral (inguinal) or bilateral (umbilicus) junctional bleeding in pigs. Acute histologic changes seen in an earlier study warranted this survival investigation.[7] The experimental model was designed to include junctional bleeding and mild hemorrhagic shock (Class II, 25% uncontrolled hemorrhage) to simulate a likely scenario in which a patient may lose a significant amount of blood before CRoC can be prepared and applied. It was also reported previously that ischemic injury in pig extremities is compounded in the presence of hemorrhagic shock.[10] We initially performed four model development experiments to examine overall effects of the surgical procedures and hemorrhage on the general recovery and mobility of the animals. The results were encouraging, and even with bilateral femoral artery injuries and repairs, the animals recovered well and were able to resume normal walking within 2 days to 3 days.

The first arm of the study investigated the inguinal application of CRoC to stop groin hemorrhage for 2 hours compared with a control group (the second arm) in which bleeding was controlled with CG. As the CT scans showed, application of CRoC totally occluded left iliac arteries and prevented any collateral blood flow to the hind leg. This total ischemia plus possible compression injuries by CRoC delayed full recovery of leg function by several (8–9) days. The average pressure needed by CRoC to stop hemorrhage was 524 mm Hg, which was considerably less than those measured (800–900 mm Hg) in the

Figure 6. Gross unilateral lumbar muscle necrosis (*arrows*) of a pig discovered during necropsy 2 weeks after 2-hour umbilical application of CRoC. This animal did not recover full leg function (Tarlov score, 3) during the 2-week observation period. Bilateral and extensive lumbar muscle necrosis was also found in another umbilicus CRoC-applied pig that had to be euthanized 3 days after the surgery.

previous study.[7] This discrepancy was because in this study, CRoC was applied on the inguinal area (above wound) directly over the iliac artery, while in the previous study, CRoC was applied on the groin wound that was covered with several layers of gauze, which dampened the CRoC's pressure on the vessel. The control-treated legs that were subjected only to a partial ischemia (femoral artery blood flow was interrupted) recovered in 3 days. Total occlusion of blood flow with CRoC may be advantageous when treating high amputation bleeding in the field, but in cases of junctional wounds, it may delay recovery of the affected limb and cause more damage (e.g., muscle necrosis) if it is left in place for extended periods (e.g., >2 hours). In this study, no muscle necrosis was found in the controls, but extensive necrosis of the peroneus muscle was seen in one CRoC animal that did not recover full mobility and had the lowest peroneus muscle strength at 2 weeks. Histologic changes in sciatic and saphenous nerves were seen in both groups but were more common in the CRoC than in the control group. These reactive changes did not seem clinically significant and should not prevent complete recovery of leg function (pathologist opinion). Tissue injury biomarkers in CRoC animals, which increased severalfold compared with the controls on Day 2, returned to baseline levels within 1 week after surgery, further indicating that the injuries by CRoC inguinal application were reversible.

The impacts of ischemia-reperfusion on neuromuscular functional recovery of a swine hind leg have been studied in a similar model in which different ischemic periods were imposed by temporary ligation of the right iliac artery and its branches.[11] In that model, up to 3-hour hind limb ischemia was tolerated, and almost 90% of neuromuscular function of the leg was restored in 14 days of recovery period.[12] However, in the presence of severe hemorrhagic shock, the ischemic threshold of the limb was significantly reduced (<3 hours), and a more rapid (≤60 minutes) restoration of blood flow was needed to improve functional salvage of an injured extremity.[10]

The third arm of this study was designed to evaluate the long-term effects of umbilicus application of CRoC to occlude the abdominal aorta, thereby preventing blood flow to both hind legs. The initial pilot experiments revealed that CRoC had to be applied with substantially higher pressure to overcome bulky intestines and to compress the abdominal aorta. Pressure from the base plate of CRoC, which pressed against the lower back, apparently damaged its circulation, resulting in gross ischemic necrosis of the lumbar muscles and permanent movement disability in two of three animals that had no blood flow in their extremities. CRoC application also caused some urogenital injuries in one pig (unable to void) that showed most disability. The use of a similar device, Lister's Abdominal Tourniquet, which was invented by Dr. Joseph Lister (1827–1912) to compress the abdominal aorta and perform bloodless operations (e.g., amputation), was abandoned after discovering that it often damaged internal organs and could cause death.[13] Given the historical evidence and the poor outcomes we found in these pilot experiments, it may be wise to avoid umbilical application of CRoC and consider using other junctional TQ devices for bilateral control of hemorrhage.

The main limitation of this study is that it was conducted in animals and the findings may not be translatable to humans. One should be reminded that pigs' hind legs are proportionally smaller and receive a smaller percentage of total blood flow compared with humans' lower extremities. Therefore, it may require less pressure and cause less tissue damage to apply CRoC to control hemorrhage in pigs than in humans. An observational study in healthy volunteers has shown that inguinal application of CRoC to interrupt popliteal blood flow for 15 seconds was more difficult and occasionally unsuccessful in heavier individuals (>100 kg) with larger thighs and high blood pressures.[14] Another limitation of this study was related to CRoC's pressure measurement, which was performed at the conclusion of the surgical procedure to avoid contamination of sterile surgical field.

The safety of CRoC must ultimately be confirmed in human subjects who are treated with this device in the field or in hospitals. Although control of hemorrhage and preventing exsanguination of an amputee patient has the highest priority, which may be better accomplished with CRoC, one also needs to be concerned about subsequent preservation and recovery of amputee's residual limb. The benefit of using CRoC must also be balanced against its potential risk in patients with other types of junctional wounds. A clinical trial to examine safety of junctional TQs in trauma patients is not feasible, and therefore, data from survival animal studies may be the best information we can gather. The available human data are anecdotal reports testifying to the efficacy of CRoC when used either in a hospital to stop moderate bleeding from a cannulation site in a cardiac patient (personal communication, John B. Holcomb, TMC, 2013) or on the battlefield to control hemorrhage in a casualty after amputation injury.[5] However, these reports provide little or no information about possible adverse effects of CRoC use.

In summary, the 2-hour inguinal application of CRoC with associated compression and ischemic-reperfusion injuries caused only mild neural and muscular damages that were reversible and allowed full recovery of voluntary leg function within 10 days in most (five of six) animals. In contrast, 2-hour umbilicus application of CRoC resulted in extensive lumbar muscle necrosis and permanent disabilities in two of four pigs. While inguinal application of CRoC seems safe and effective, umbilicus application may not be and could cause irreversible injuries. Therefore, for bilateral control of junctional hemorrhage, alternative junctional TQs should be considered.

AUTHORSHIP

All authors contributed in designing the study, developing methodology as well as interpretation and editing of the manuscript. B.S.K. with the technical assistance of I.B.T. and N.M. contributed in the surgical procedures, treatments, data collection and analysis, as well as manuscript preparation. J.S.E., a veterinarian pathologist, performed the histologic analysis. B.T.C. performed the neuromuscular function measurements. J.F.K. trained and supervised the device (CRoC) application. M.A.D. guided the study and edited the manuscript.

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DISCLOSURE

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